

LISTING OF THE CLAIMS

1. (Previously Presented) A method of assembling several DNA units in sequence in a DNA construct, which method comprises the steps of

a) providing each DNA unit with a restriction enzyme recognition sequence at its 5' end and with a recognition sequence for the same restriction enzyme at its 3' end that is combined with a recognition site for a DNA modification enzyme.

b) providing a starting DNA construct having an accessible restriction site for the same or a compatible restriction enzyme and cleaving the starting DNA construct with such a restriction enzyme,

c) inserting the desired DNA unit and bringing the ligated product into contact with a DNA modification enzyme such that the restriction site at the 3' end of the inserted DNA unit is abolished

d) cleaving the ligated product at an accessible unmodified recognition site for the same or a compatible restriction enzyme,

e) repeating steps c) and d) to introduce each desired DNA unit to give a DNA construct containing all the desired units in sequence.

2. (Previously Presented) The method of claim 1 wherein the DNA modification enzyme is a methylase.

3. (Previously Presented) Previously Presented The method of claim 2 wherein the methylase is the dam methylase of *Escherichia coli*.

4. (Previously Presented) A method of assembling several DNA units in a DNA construct which method comprises the steps of

a) providing each DNA unit with an *Xba*I recognition sequence 5'XXTCTAGA3' (where XX is not GA) at its 5' end and with an *Xba*I recognition sequence 5'GATCTAGA3' at its 3' end,

b) providing a starting DNA construct having an accessible *Xba*I site and cleaving the starting DNA construct with *Xba*I,

- c) inserting the desired DNA unit and using a resulting ligated product to transform a *dam*⁺ strain of *E. coli*,
- d) recovering a resulting plasmid and cleaving the plasmid at an accessible *Xba*I site with *Xba*I,
- e) repeating steps c) and d) to introduce each desired DNA unit to give a DNA construct containing all the desired units in sequence.

5. (Previously Presented) The method of any one of claims 1 to 3, wherein the recognition sequences for the restriction enzyme and the DNA modification enzyme are created in the DNA units prior to cutting with the restriction enzyme.

6. (Previously Presented) The method of claims 1 to 4 wherein the restriction sites are created in the fragment by means of a primer extension reaction.

7. (Previously Presented) The method of any one of claims 1 to 4, wherein the DNA construct is an expression vector capable of facilitating expression of the protein encoded by the desired DNA units.

8. (Previously Presented) The method of claim 3, wherein the DNA modification is removed and the restriction site re-established by replicating the ligated product in a *dam*⁻ strain of *E. coli* by means of a suitable vector.

9. (Previously Presented) A method of making an assembly of several DNA units in sequence which method comprises the steps of:

- a) providing a first DNA unit with a recognition sequence for a first restriction enzyme at its 3' end, and cleaving the said first DNA unit with said first restriction enzyme,
- b) providing each other DNA unit with a recognition sequence at its 5' end for a second restriction enzyme which has a compatible ligation sequence with that of the first restriction enzyme, and a downstream recognition sequence for said first restriction enzyme followed by a downstream recognition sequence for a third restriction enzyme at its 3' end, and cleaving each said other DNA unit with the second and third restriction enzymes,

c) ligating the said first DNA unit with a desired other DNA unit to form a ligated product such that the ligation of the two units abolishes the recognition site for the first restriction enzyme at the ligation junction, and cleaving the ligated product with said first restriction enzyme,

d) ligating the product from c) with a desired DNA unit from b) to form a ligated product and cleaving the ligated product with said first restriction enzyme

e) repeating step d) with each other DNA unit in turn so as to assemble the DNA units in sequence.

10. (Previously Presented) A method of making an assembly of several DNA units in sequence which method comprises the steps of:

a) providing a first DNA unit with an *Xba*I recognition sequence 5'TCTAGA3' at its 3' end, and cleaving the said first DNA unit with *Xba*I,

b) providing each other DNA unit with a *Spe*I recognition sequence 5'ACTAGT3' at its 5' end, and downstream *Xba*I recognition sequence 5'TCTAGA3' followed by a downstream *Sma*I recognition sequence 5'CCCGGG3' at its 3' end, cleaving each said other DNA unit with *Spe*I and *Sma*I, and dephosphorylating the 5' end of the cleaved DNA unit,

c) ligating the said first DNA unit with a desired other DNA unit to form a ligated product and cleaving the ligated product with *Xba*I,

d) ligating the product from c) with a desired DNA unit from b) to form a ligated product and cleaving the ligated product with *Xba*I

e) repeating step d) with each other DNA unit in turn so as to assemble the DNA units in sequence.

11. (Previously Presented) The method of claim 9 or claim 10 wherein the assembly occurs *via* stepwise addition of at least one DNA unit to a vector.

12. (Previously Presented) The method of claim 9 or claim 10 wherein the said first DNA unit is attached to the solid phase for use in step c).

13. (Previously Presented) The method of claim 12, wherein the solid phase is split and mixed between steps c), d), and e) to make several different assemblies.

14. (Previously Presented) The method of claim 9 or claim 10, wherein the recognition sequences in one or more of the DNA units are introduced by means of extension primers.

15. (Previously Presented) The method of claim 9 or claim 10, wherein the assembly of several DNA units is inserted in to an expression vector which is used to transform a host capable of expressing the protein encoded by the vector.

16. (Previously Presented) The method of any one of claims 1, 4, 9, or 10, wherein one or more of the DNA units encodes a catalytic or transport protein domain.

17. (Previously Presented) The method of claim 16 wherein one or more of the DNA units are derived from polyketide synthesising enzyme domain DNA sequences.

18. (Previously Presented) The method of claim 16 wherein one or more of the DNA units are derived from peptide synthesising enzyme domain DNA sequences.

19. (Previously Presented) The method of claim 16 wherein one or more of the DNA units are derived from hybrid peptide polyketide enzyme domain DNA sequences.

20. (Previously Presented) The method of claim 16 wherein one or more of the DNA units are derived from fatty acid synthesizing enzyme domain DNA sequences.

21. (Previously Presented) The method of claim 16 wherein one or more of the DNA units encode modules comprising one or more catalytic or transport domains.

22. (Previously Presented) A DNA construct incorporating one or more DNA assemblies encoding synthetic enzymes made by any one of the methods of claims 1, 4, 9, or 10.

23. (Previously Presented) A synthetic enzyme encoded by one or more DNA assemblies made by the methods of anyone of claims 1, 4, 9, or 10.

24. (Previously Presented) A plurality of host cells expressing DNA constructs encoding one or more synthetic enzymes made by any one of the methods of claims 1, 4, 9, or 10.

25. (Previously Presented) A plurality of transformed hybrid host cells expressing one or more DNA constructs encoding synthetic enzymes incorporating a DNA assembly made by any one of the methods of claims 1, 4, 9, or 10.

26. (Previously Presented) A compound produced by a synthetic enzyme encoded by a DNA assembly made by any one of the methods of claims 1, 4, 9, or 10.

27. (Previously Presented) A method of synthesizing a target molecule comprising the steps of

- a) examining the composition and stereochemistry of a target molecule,
- b) determining which catalytic and transport domains need to be present in a synthetic enzyme in order to catalyze the synthesis of the target molecule,
- c) using any one of the methods of claims 1, 4, 9, or 10 to assemble the required DNA units encoding the catalytic and transport domains into a DNA assembly that encodes said synthetic enzyme which is capable of synthesizing the target molecule.
- d) placing the DNA assembly into a vector to allow expression of the synthetic enzyme in a host capable of synthesizing the target molecule after transformation with said vector.

28. (Previously Presented) The method of claim 27 wherein the transformed host is tested for the presence of the target molecule after step d).

29. (Previously Presented) The transformed host of claim 27.

30. (Previously Presented) A method for producing a target molecule using the transformed host of claim 27.

31. (Previously Presented) A method of making a synthetic enzyme to catalyze the synthesis of a target molecule comprising the steps of

- a) examining the composition and stereochemistry of a target molecule,
- b) determining which catalytic and transport domains need to be present in the synthetic enzyme in order to catalyze the synthesis of the target molecule.
- c) using any one of the methods of claims 1, 4, 9, or 10 to assemble the required DNA units encoding the catalytic and transport domains into a DNA assembly that encodes an enzyme which is capable of synthesizing the target molecule.
- d) expressing the DNA assembly in a suitable host to produce the enzyme.

32. (Previously Presented) A library of DNA units encoding catalytic or transport protein domains, wherein each DNA unit has a recognition sequence for a restriction enzyme at its 5'-end and a second recognition sequence for the same or a compatible enzyme at its 3'-end which incorporates a recognition sequence for a DNA modifying enzyme.

33. (Previously Presented) The library of claim 32, wherein each DNA unit has an *Xba*I recognition sequence 5'X₂TCTAGA3' (where X₂ is not GA) at its 5'-end and an *Xba*I recognition sequence 5'GATCTAGA3' at its 3'-end.

34. (Previously Presented) A library of DNA units encoding catalytic or transport protein domains, wherein each DNA unit has a recognition sequence at its 5' end for a first restriction enzyme, and a downstream recognition sequence for a second restriction enzyme followed by a downstream recognition sequence for a third restriction enzyme at its 3' end, such that the DNA units, once restricted by the first and second restriction enzymes can be ligated together to abolish the restriction sites at the ligation junction.

35. (Previously Presented) The library of claim 34, wherein each DNA unit has a *Spe*I recognition sequence 5'ACTAGT3' at its 5'-end, and a downstream *Xba*I recognition sequence 5'TCTAGA3' followed by a downstream *Sma*I recognition sequence 5'CCCGGG3' at its 3'-end.

36. (Previously Presented) The library of claim 32 or claim 34, wherein the DNA units encode polyketide synthetic domains, comprising two KS domains, at least two AT domains, two KR domains, two DH domains, two ER domains, an ACP domain and a TE domain.

37. (Previously Presented) A module comprising a DNA sequence encoding a functional set of polyketide synthetic domains wherein the module has a recognition sequence for a restriction enzyme at its 5'-end and a second recognition sequence for the same or a compatible enzyme at its 3'-end which incorporates a recognition sequence for a DNA modifying enzyme.

38. (Previously Presented) The module as claimed in claim 37, wherein the module has an *Xba*I recognition sequence 5'XXTCTAGA3' (where XX is not GA) at its 5'-end and an *Xba*I recognition sequence 5'GATCTAGA3' at its 3'-end.

39. (Previously Presented) A module comprising a DNA sequence encoding a functional set of polyketide synthetic domains wherein the module has a recognition sequence at its 5' end for a first restriction enzyme, and a downstream recognition sequence for a second restriction enzyme followed a downstream recognition sequence for a third restriction enzyme at its 3' end, such that the DNA units, once restricted by the first and second restriction enzymes can be ligated together to abolish the restriction sites at the ligation junction.

40. (Previously Presented) The module as claimed in claim 39, wherein the module has a *Spe*I recognition sequence 5'ACTAGT3' at its 5'-end, and a downstream *Xba*I recognition sequence 5'TCTAGA3' followed by a downstream *Sma*I recognition sequence 5'CCCGGG3' at its 3'-end.

41. (Previously Presented) A module as claimed in claim 37 or claim 39, wherein the DNA units encode polyketide synthetic domains, comprising two KS domains, at least two AT domains, two KR domains, two DH domains, two ER domains, an ACP domain and a TE domain.

42. (Previously Presented) A vector containing one or more modules as claimed in claim 37 or claim 39.

43. (Previously Presented) The vector as claimed in claim 42, wherein a non-functional *recA* gene is also present.

44. (Previously Presented) A method of transforming a host with one or more synthetic DNA assemblies encoding enzyme domains which comprises the steps of:

- a) inserting said DNA assembly into a vector containing a mutated internal fragment of a *recA* gene sequence such that the vector is capable of undergoing homologous recombination with the *recA* gene of the host,
- b) bringing said vector into contact with a host chromosome under conditions which permit homologous recombination to take place,
- c) disrupting the host *recA* gene by the integration of the DNA of said vector into the chromosome.

45. (Previously Presented) The method of claim 44 wherein the expression vector is used to transform a *Streptomyces* host.

46. (Previously Presented) The method of claim 44 or claim 45, wherein the DNA assemblies are modules according to claim 35 or claim 37.

47. (Previously Presented) A host lacking a *recA* function, transformed with a vector containing one or more modules according to claim 35 or 37.

48. (Previously Presented) A kit containing an element selected from the group consisting of DNA units, DNA modules, vectors, DNA manipulation hosts, DNA modification hosts, expression hosts, and solid phase elements for use in any one of the methods of claims 1, 4, 9, 10, or 44.